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Agricultural Research

The New Technologies—What They Mean to Agriculture

Because American farmers—only 3 percent of our population—feed this Nation abundantly and provide 70 percent of the food aid to food-deficient countries, the obstacles to increased agricultural productivity hardly seem obvious or even particularly necessary to overcome.

Yet there is so much we do not know about the basic biological processes of our crops and livestock. And if we knew more, we could make enormous improvements not only in production levels, but also in farming practices to conserve and protect our soil, water, and other resources.

For example, up to 35 percent of the total productive capacity of all crops is now accredited to the application of one element—chemically fixed nitrogen. Almost one-third of all fossil fuel now used in agricultural production goes to chemical nitrogen fertilizer synthesis. The United States annually consumes about one-fourth of the world's supply of nitrogen fertilizer, half of which goes into a single crop—corn. If corn and other important nonleguminous crops could fix their own nitrogen from the soil, the impact on fuel supplies and economics worldwide would be enormous.

Improvements such as this appear to be a long way off, however. Some may prove to be impossible dreams. But two relatively new technologies—genetic engineering and monoclonal antibody techniques—are bringing about some advancements much more quickly than we could have predicted as little as 5 years ago.

Because of the early successes in using genetic engineering to clone proteins that are in short supply from natural sources, this aspect of the technology is now at center stage. Products that have been produced by this method include hormones, enzymes, interferons, and vaccines. A recent example of success in this area is USDA's announcement last summer of the development of a technique to produce a safe, effective, and relatively inexpensive vaccine for foot-and-mouth disease.

Another advance—a technology for moving genes from one species of plant to another—lays the groundwork for dramatic improvements in many areas of crop production. Although we have been able to transfer genes between plants of the same species, not until now has interspecies transfer been possible. Now the challenge is to convert the genetically changed tissue into a plant that will bear seed, and discover what effect the transferred gene has on the characteristics of the new species.

The major goals generally envisioned for plant genetic engineering include:

- introducing nitrogen-fixing ability into corn and such cereal crops as wheat, barley, and rye;
- increasing the photosynthetic efficiency of plants;
- increasing the resistance of all crop plants to diseases, herbicides, temperature extremes, drought, and soil salinity;
- and increasing the ability of microbes to convert agricultural biomass into fuel and single-cell proteins.

Transformations that can be effected through single-gene splicing have the greatest prospect for early success. Fortunately, these include the resistance of plants to a wide variety of organisms, including fungi, viruses, nematodes, bacteria, mycoplasmas, and insects, which together consume 25 to 35 percent of American crops each year.

There may be a dozen single-gene transplants of potential benefit to agriculture that could be achieved in the next 3 to 5 years. Some of these would enable crop plants to make their own preventive medicines. For example, if the gene from a soil bacterium that degrades herbicides could be transplanted to crop plants, the crop would become resistant to the herbicide, leaving only the undesirable weeds vulnerable to chemical treatment.

Transformations that would require multiple-gene insertions—to increase photosynthetic efficiency, for example—will be more difficult, and take longer, to accomplish. The complex process of photosynthesis is controlled by several genes. To find them and understand how the genes are turned on and off in concert with the other activities of the plant's growth and development will require lengthy basic research.

But the fruits of this research are enormous. For example, scientists theorize that plants can use up to 12 percent of the sun's energy available to them for photosynthesis. Yet even in the best farm operations in the world, crops now use less than 1 percent. Even a modest increase in photosynthetic efficiency could significantly boost crop yields.

The second exciting new technology—monoclonal antibody—is applicable to an enormously diverse range of research. It can tell scientists, much more quickly than before, if an animal has been vaccinated against a disease or if it has actually been infected. And in the process, antibodies are produced which are a vital first step in developing vaccines against the disease being diagnosed. This approach is making steady headway against such costly diseases as mastitis in dairy cows, coccidiosis in chickens, and bluetongue in sheep.

Monoclonal antibody techniques are also probing the secrets of how plant viruses function. Progress in understanding plants' immune systems, separating out plant toxins, and imparting disease resistance to plants through recombinant DNA techniques is being furthered by the knowledge stemming from monoclonal antibody techniques.

In short, these technologies will allow scientists to explore and use the hereditary material found in every plant and animal species as it never has been before.

The Agricultural Research Service is addressing high-risk/high-potential areas for top research priorities as well as areas on the threshold of a breakthrough for new technology.

Genetic engineering and monoclonal antibody technology will augment our continuing work in quantitative genetics. Through agricultural research—and with a little luck—we can hope to meet the challenge of doubling our food production by the second or third decade of the next century.

Mary E. Carter
Acting Administrator, ARS

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Cover: A representation of the DNA double helix comprising the genes that direct reproduction, growth, and life. Lengthy and painstaking research into the genetic material in plant and animal germplasm will in time unlock these vast resources to the benefit of all agriculture. (A special section on genetic engineering in agriculture begins on page 8.) (PN6858)

"Agroleum" Fuels

The adage that "oil and water don't mix" may have to be modified somewhat if experiments at the Northern Regional Research Center, Peoria, Ill., continue to prove successful.

Arthur W. Schwab, ARS vegetable oils chemist, has developed formulas to combine aqueous ethanol (ethyl alcohol containing water) with either gasoline or diesel oil in microemulsions that remain clear and stable at subfreezing temperatures. The result is an "agroleum" fuel—so called because it is a hybrid of agricultural and petroleum products.

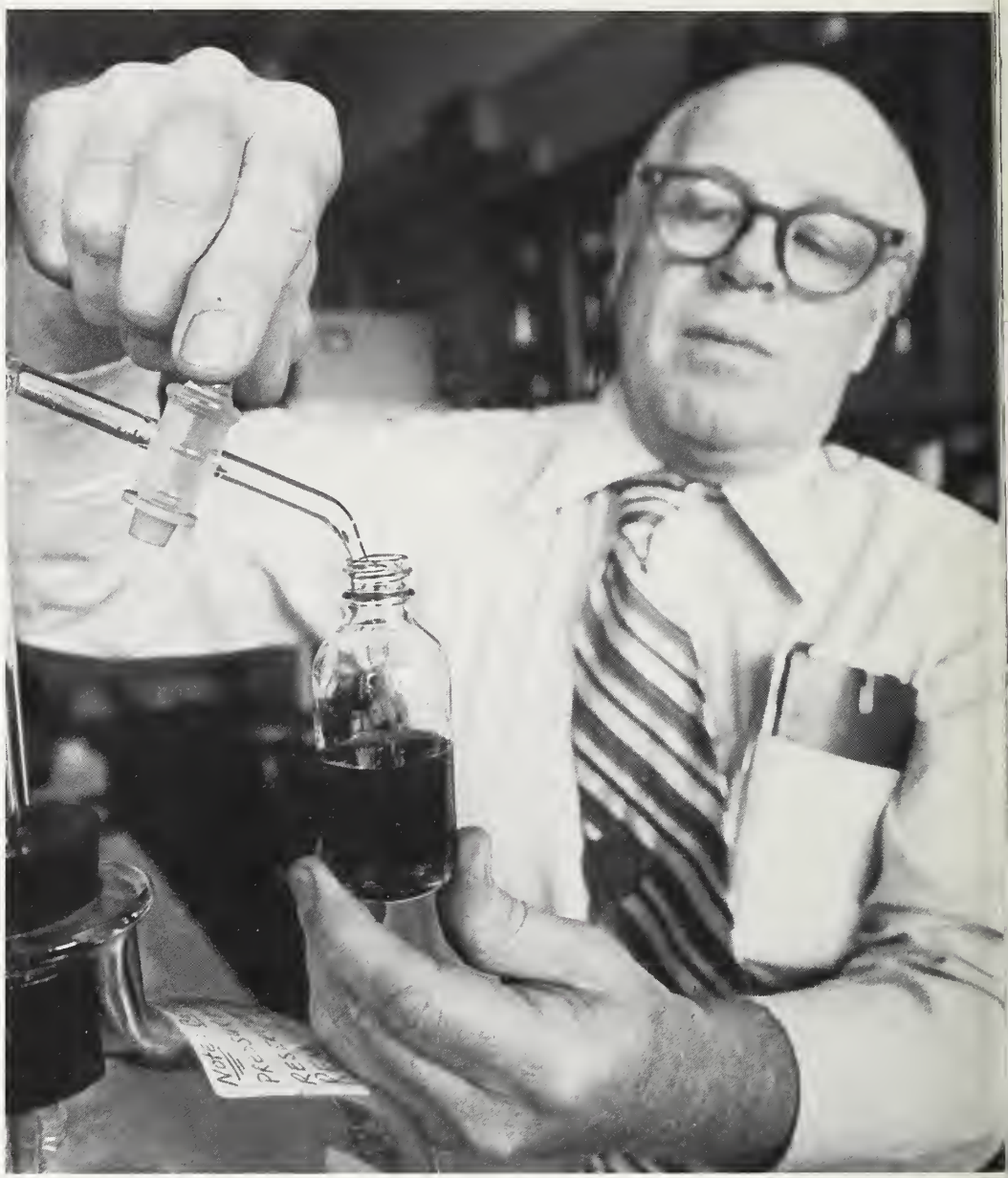
These "agroleum" fuels would cost 30 to 77 percent more than petroleum fuels at current prices, estimates Everett H. Pryde, research leader for the project, but cost differences would drop if petroleum prices increased more than farm product prices.

A persistent problem in mixing ethanol distilled from agricultural products with petroleum fuel is that ethanol contains water unless it undergoes a special distilling process. Even pure ethanol absorbs water from the air. Ordinary mixtures of aqueous ethanol and petroleum tend to separate and cause fuel problems in engines.

If kept from separating, however, the water present in "agroleum" fuels might offer some advantages. It could add a safety factor to aviation and military fuels, release fewer pollutants when burned, lower engine temperatures, and reduce engine carbon buildup.

The microemulsions tolerate the water in ethanol without separating, according to Schwab, because they contain either butanol or fatty acids acting as a soap or surfactant. Surfactants—surface active agents—reduce the molecular tension that acts like an elastic skin at the surface of a liquid or at the boundary between liquids that do not mix.

Ethanol, butanol, and fatty acids can all be made from farm products, Schwab points out. Fatty acids are produced from such oil crops as soybeans, sunflowers, and peanuts. Ethanol and butanol are fermented from plant sugars such as corn glucose, and distilled for purity. "Agroleum" fuel components, possibly including farm-produced ethanol, could potentially be mixed right on the farm.



Vegetable oils chemist Arthur W. Schwab releases alcohol, which naturally contains water, into a vial of diesel fuel. Schwab's formulas keep the water from separating and causing fuel problems in engines. (0881X1008-6)

In formulas using fatty acids as a surfactant, Schwab combines the acids with ammonia derivatives, called amines. Together, the amines and fatty acids form a kind of water-soluble soap. Each soap molecule has an amine head that is attracted to water, and a fatty acid tail. In an aqueous ethanol-petroleum fuel mix, the soap molecules are drawn to the water-oil interface and the fatty acid tails fan out in all directions. The soap molecules form the water into submicroscopic, spherical droplets and surround them, and the ethanol and oil molecules probably wedge in among the fatty acid tails, spontaneously forming the microemulsion, Schwab explains.

The soap-encapsulated water droplets are so small that they are not visible under a light microscope. Constantly bombarded in the mixture by the large, hydrocarbon fuel molecules, the droplets bounce around in a random motion and do not coalesce.

Microemulsions of aqueous ethanol in gasoline are clear and almost colorless. Aqueous ethanol in diesel fuel makes a clear, red-orange liquid, "the color of a sunset," Schwab says. Color and clarity indicate that the water-droplet size is the proper smallness, and that the microemulsion is stable.

Each formula has a particular low temperature at which it loses stability and becomes cloudy. When warmed to above that critical temperature, the microemulsion clears spontaneously.

To make an "agroleum" fuel that remains stable at a specified low temperature, Schwab increases the ratio of surfactant to water in the ethanol. Especially in formulas containing diesel oil, however, the difficulty is in keeping the experimental fuel thin enough to flow into and lubricate parts of the fuel injector pumps used now on some engines.

Early formulas for 190-proof ethanol in diesel oil "largely meet the viscosity requirement," Schwab says, "but formulas with 160-proof ethanol were only partially successful."

One formula that meets the viscosity requirement and has been engine tested is composed of 1 gallon of 190-proof ethanol (5 percent water) in 2 gallons of diesel oil, using butanol as a surfactant. The microemulsion remained stable at −18°C (0°F). Agricultural engineers at the University of Illinois ran engine tests in cooperation with Schwab.

Microemulsions containing more of the agricultural product could be tested as "agroleum" fuels, Schwab says, if engine parts were designed for a wider range of fuel properties.

Schwab has tried other microemulsion formulas—one of 160-proof ethanol (20 percent water) in gasoline, and another of 166-proof ethanol (17 percent water) in diesel oil. The first remained stable at −30°C (−22°F), and the second remained stable at −16°C (3°F) in laboratory tests, but neither has been engine tested.

The bottle on the left shows aqueous alcohol floating on top of diesel oil. Using butanol as a surfactant, the two liquids emulsify (right) and the water does not separate. (0881X1008-15)

In comparing the stability of diesel oil microemulsions containing added amines to those made without, Schwab says the amines "appreciably improve water tolerance." Either fatty acids or butanol alone gives less water tolerance in the microemulsion.

"Chemistry of the mixed film is still not completely understood," says Schwab. "We're just scratching the surface in microemulsions as hybrid fuels and their applications to energy problems."

Arthur W. Schwab is located at the Northern Regional Research Center, 1815 University St., Peoria, IL 61604.—(By Dean Mayberry, Peoria, Ill.) ■

Quick Test for Goat and Sheep Disease

Researchers at Pullman, Wash., have developed the first quick, reliable, and economical test for caseous lymphadenitis, a major worldwide disease of goats and sheep.

A derivation of the ELISA (enzyme-linked immunosorbent assay) virus detection technique already in use against human and plant diseases, the new test will be used to help maintain disease-free flocks and also as a research tool.

Microbiologist David T. Shen, along with veterinarians John R. Gorham and L. W. Jen, developed the test which, in its first version, applies only to goats: caseous lymphadenitis is the number one goat disease in the United States.

Caseous lymphadenitis is a disease that involves the lymph nodes. Abscesses are found internally and on various parts of an infected animal's body. In goats, milk production is reduced and the animal can die from it. The disease is known to be transmitted by skin contact through wounds and abrasions but it is suspected that there are additional means of transmission.

Screening animals for infection before introducing them to a disease-free herd has always been impractical because the incubation period of caseous lymphadenitis is 1 to 2 months. Several laboratory diagnostic tests have been available but these tests lacked accuracy and sensitivity, and were too costly and complex.

The ARS researchers used their ELISA test in a recent survey of goat herds in Washington and Texas to show that in goats, the occurrence of caseous lymphadenitis increases with age. The researchers are now adapting the test to sheep.

David T. Shen is located in the Veterinary Science Building, Washington State University, Pullman, WA 99164.—(By Lynn Yarris, Oakland, Calif.) ■

Harnessing the Potato's Genetic Diversity

Geneticist Robert E. Hanneman, Jr., examines flowers of a meiotic mutant potato plant. A special characteristic of this and other mutants allows crossbreeding with commercial potato varieties. (0382X247-27)

Research technician Richand Rhude prepares slides for microscopic studies of pollen from a meiotic mutant potato plant. (0382X247-4)

Using fertile mutants of South American potato varieties, scientists have found a way to tap the great genetic diversity within the species *Solanum tuberosum*. The technique will help make desirable characteristics in wild South American varieties available for crossbreeding into U.S. commercial varieties. Resistance to disease, insects, and frost, better nutritional composition, and substantial yield increases are some of the improvements that may result.

Crossbreeding between wild South American and commercial potato varieties has been hampered because almost 70 percent of wild potatoes have two sets of chromosomes (diploid), whereas commercial potatoes have four sets (tetraploid).

Meiotic mutants of South American varieties hold promise as a means of bridging that chromosome gap. Their germ cells do not undergo the normal kind of cell division that keeps the number of chromosomes constant from one generation to the next. Instead, certain meiotic mutants have been found to have a gene that causes parallel spindles to form in chromosomes in male pollen, thus doubling the normal number to four. About 80 percent of a set of doubled chromosomes can be transmitted intact in crossbreeding between the South American diploid and commercial tetraploid lines.

ARS plant geneticist Robert E. Hanneman, Jr., and University of Wisconsin-Madison plant geneticist Stanley J. Peloquin plan to incorporate the mutants into a conventional potato breeding program. Their experiments have already had a positive effect on yields. "We have obtained some tetraploid families that yield more than standard commercial varieties," says

Hanneman. "As breeders learn how to select the best parents from a broadened genetic base for these crosses, we would not be surprised to see yields increased 20 to 30 percent."

Hanneman says he believes the favorable yields they have already observed are due to heterosis—hybrid vigor—that was passed by the pollen to the offspring.

Hanneman and Peloquin are also using another mutant called a meiotic synaptic variant. They have identified fertile clones of this mutant that also carry the gene for chromosome doubling, and that can transmit 100 percent of a set of chromosomes from the male pollen intact when crossbred. Without the parallel spindles characteristic, however, pollen from a meiotic synaptic variant is completely infertile.

"We're finding these fertile mutants provide us with an efficient method for capturing the genetic diversity of wild potatoes and transferring the germplasm essentially intact to the tetraploid level," Hanneman says.

Harnessing the genetic diversity of the species may lead to potato farming in an extended range of geographic areas. Already, potatoes are the most important vegetable crop in the world. On a per-acre basis, Hanneman says, potatoes have the greatest potential of all major food crops for producing maximum nutrients—carbohydrates and proteins.

Research on potatoes with different chromosome number levels is expected to lead to improved understanding of genetic inheritance. Diploid inheritance patterns are much simpler than tetraploid patterns, says Hanneman.

Hanneman envisions a reservoir of diploid meiotic mutant stocks that plant breeders could use to raise conventional yield ceilings. The stocks could also be screened for disease and pest resistance and be available quickly for crossbreeding programs to overcome new disease outbreaks.

Robert E. Hanneman, Jr., is located at the Horticulture Department, and Stanley J. Peloquin at the Genetics Department, University of Wisconsin-Madison, Madison, WI 53706.—(By Ben Hardin, Peoria, Ill.) ■

Artificial Diet Feeds Bees

Ten years of bee nutrition research have produced an inexpensive artificial diet for honeybees that makes life easier around the hive and makes beekeeping more profitable.

Released earlier this year, the Beltsville Bee Diet, developed at the ARS Bioenvironmental Bee Laboratory, Beltsville, Md., allows colonies of bees to build up their populations faster and earlier in the season. The broods are then ready to pollinate such early spring crops as blueberries and almonds, and they start producing honey earlier in the season.

Artificial diets are also needed when bees are confined during extreme weather and when colonies are isolated from areas of pesticide spraying. In addition, the Beltsville Bee Diet is a foolproof way to avoid transmitting diseases to a colony through bee food.

Bee Lab scientists Elton W. Herbert and Hashiro Shimanuki started the search for the right formula by identifying the exact nutrients honeybees require to reproduce efficiently. "Then, we faced the perhaps more difficult task of making the bees eat it. We mixed and matched many ingredients until we hit on a diet that not only serves honeybees what we know they need, but also what they want to eat," says Herbert.

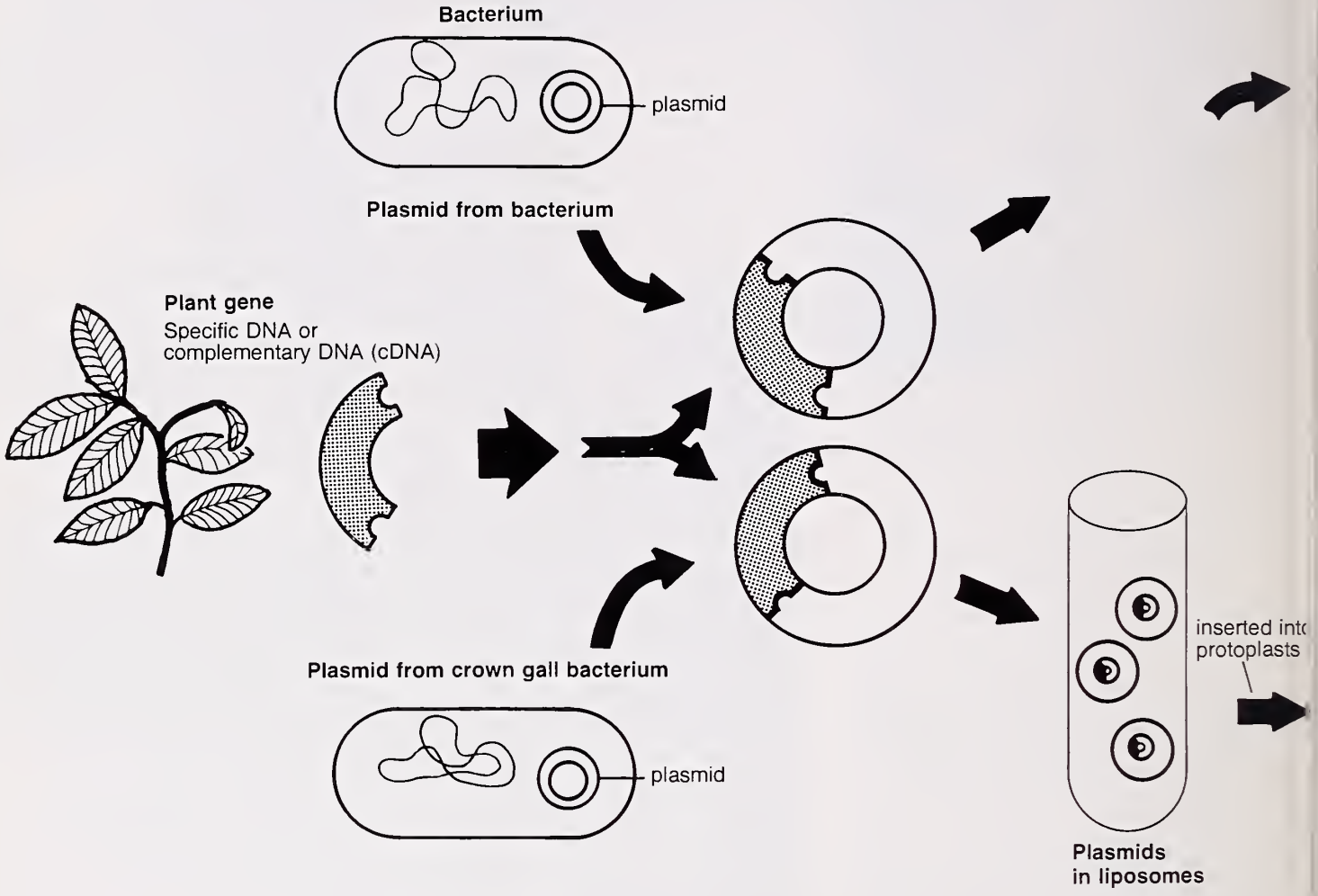
To fill the critical protein requirement—the pollen substitute—the scientists settled for a whey-yeast compound. Whey is a byproduct of cheese-making. The yeast provides honeybees with needed vitamins and minerals. Sugars in the diet provide carbohydrates, which also help preserve the formulation.

The Beltsville Bee Diet can be kept at room temperature for many months without spoiling or hardening. It has the color and viscosity of peanut butter.

Elton W. Herbert and Hashiro Shimanuki are located in Bldg. 476, Beltsville Agricultural Research Center-East, Beltsville, MD 20705.—(By Stephen Berberich, Beltsville, Md.) ■

Entomologists Elton W. Herbert (left) and Hachiro Shimanuki inspect a flat of Beltsville Bee Diet completely devoured by bees. On the open hive lie a scarcely touched soybean diet (left) and a partially eaten pollen-based diet. (0482X388-20)

Vectors or Carriers



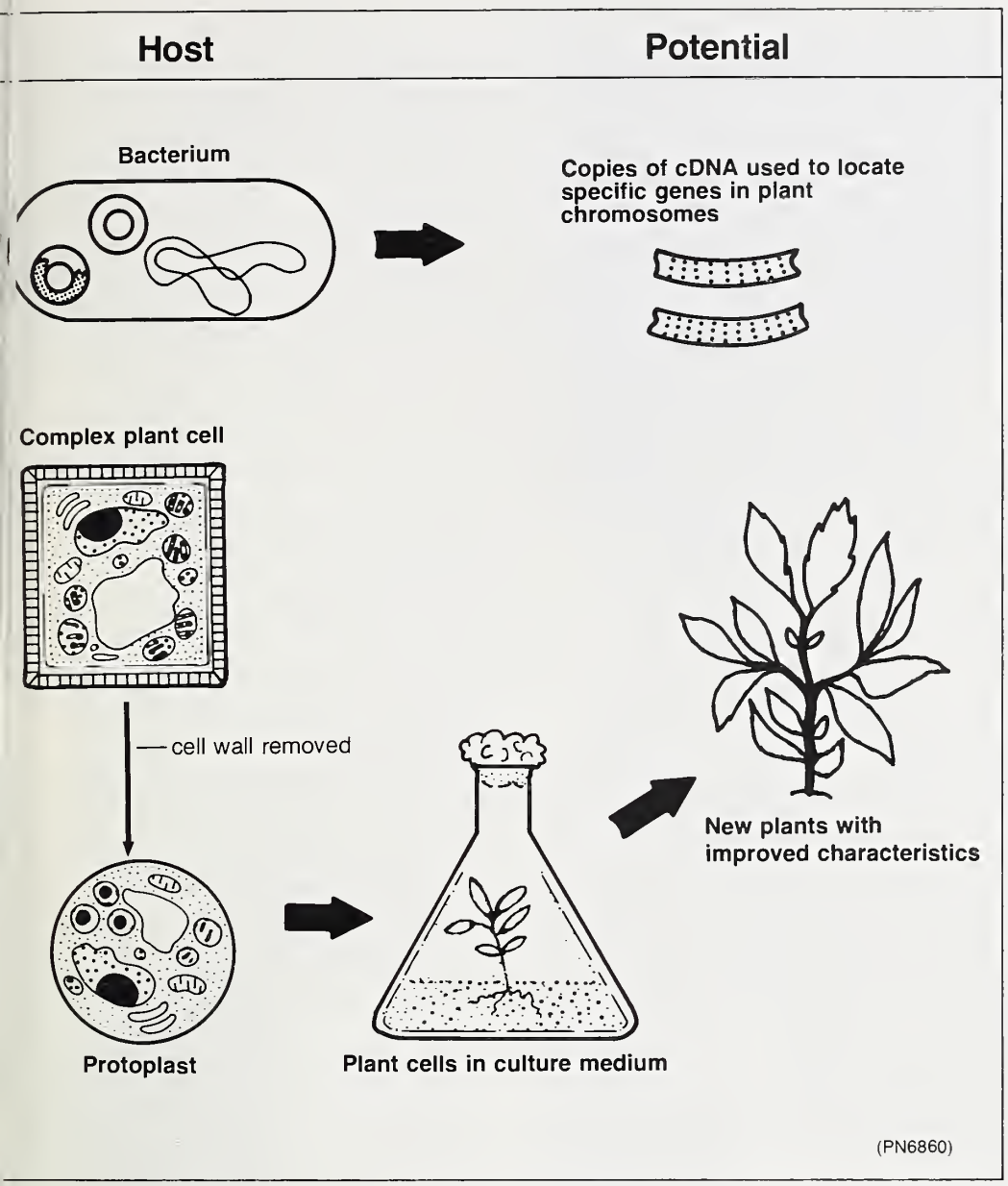
The diagram above illustrates, in a simplified manner, some genetic engineering techniques scientists are using in research to improve crop plants.

On the left, the diagram represents a gene removed from a plant. However simple this may seem, it requires a complicated laboratory procedure which plant geneticists are still working to understand and improve.

Crop plants are genetically complex, with many chromosomes enclosed inside each cell's nucleus. Desirable traits—increased productivity, disease resistance, and others—may often be controlled by several genes located on different chromosomes, unlike the more simple, single-ringed chromosome of the bacteria used in genetic engineering in the pharmaceutical and other industries.

To locate a specific plant gene, scientists must first identify it in the chromosomes in the nucleus. To do this, they isolate specific messenger ribonucleic acid (mRNA) from plant cells. This specific mRNA is used by scientists as a template for building a piece of complementary DNA (cDNA)—almost a mirror image of the mRNA. (See "Super Seeds," this issue.) They then splice the cDNA into a plasmid—the ring of genetic material which they have removed from a bacterium. Plasmids from *E. coli* and other bacteria are becoming increasingly important as carriers, or vectors, for transferring genes from one plant species to another.

The plasmid containing the cDNA is reinserted into the bacterium, where it replicates as the microorganism reproduces, creating many copies of the cDNA. Scientists then chemically label the cDNA copies and use them as probes. The labeled cDNA will attach itself to its twin DNA in the plant



chromosome, showing the exact location of the specific gene.

The lower portion of the diagram illustrates how plasmids from crown gall bacteria (*Agrobacterium tumefaciens*) are used to transfer plant genes located and identified by the procedure just described. *Agrobacterium* are the only bacteria known to transfer genes to the plants they infect. (See "The 'Gall' to Cross Mother Nature," this issue.) However, the disease-producing characteristics of the *Agrobacterium* must be overcome if it is to be useful in the long term as a genetic transfer agent.

Before a vector can penetrate a complicated plant cell, the latter's tough cellulose covering must be removed. Scientists use enzymes to digest the cell wall, leaving only a thin, flexible membrane enclosing the inner cell parts. These wall-less cells are called protoplasts.

To transfer a specific plant gene to a new plant, scientists splice the gene into the *Agrobacterium* plasmid. To form a protective shield around plasmids and assist them in entering the cell protoplasts, scientists mix the plasmids with fatty acid derivatives. Tiny fat bubbles form, each with a plasmid in the center. These fat bubbles are called liposomes.

The plasmid-containing liposomes enter the plant cell protoplasts when they are mixed together. The genetically altered protoplasts are placed in a culture medium with nutrients and plant hormones to grow.

In order for agriculture to benefit from genetic transfers, a mature plant—complete with flowers and fruits—must be regenerated from the protoplasts. Scientists have been able to grow whole plants from some genetically altered protoplasts, but many crop plants cannot yet be obtained from these single cells. Scientists must also learn how to control plant gene expression—how to turn plant genes on and off. Without this knowledge, when a gene is moved from one plant to another, it may not function properly.

Several other methods for altering the genetic makeup of cells also fall under the broad category of genetic engineering. One is cell fusion—a process in which protoplasts from two genetically incompatible plants are fused and grown into adult plants using tissue culture techniques.

Another method—anther culture—allows scientists to grow complete plants from only the male flower parts. Nutritionally improved rice and better wheat have been grown successfully using anther culture. (See "Test-Tube Rice Yields Better Protein" and "Anther Culture Speeds Up Wheat Breeding," this issue.)

This section of the magazine contains articles on ARS genetic engineering research reported at the Beltsville Symposium on genetic engineering. The titles listed immediately following will refer you to ARS genetic engineering research previously published in the magazine.

- "Recombinant DNA Techniques for Vaccine Production," June 1980, p. 5
- "Gene-Splicing Prevents Potato Losses," October 1981, p. 8
- "Bean Gene Moved to Sunflower Cell," August 1981, p. 4
- "Plasmids: New Key to Genetic Engineering?" June 1982, p. 8.

(By Ellen Mika, Beltsville, Md.) ■

Super Seeds

Research chemist G. Ram Chandra (right) and University of Maryland graduate student George Albough assess the uniformity and vigor of barley having good germination ability. (0782W696-8A)

Recombinant-DNA technology may help unravel the riddle of why some seeds sprout and others do not.

ARS scientists have found that the seed's nutrients are unlocked for germination by cells in the tissue layer that encases them. This layer is called the aleurone. In barley, rice, and wheat seeds, a hormone called gibberellic acid, produced by the plant embryo inside the seed, triggers aleurone cells to produce enzymes to digest the stored proteins and starches. The resulting simple sugars and amino acids supply the developing embryo with energy to germinate and grow.

If scientists could clone the genes that respond to the hormone trigger and produce a uniform supply of nutrients, this could lead to the production of "super" seeds with increased vigor and the ability to germinate uniformly, according to G. Ram Chandra, chief of the Seed Research Laboratory at the Agricultural Research Center, Beltsville, Md. "Uniform germination would initiate uniform growth and ripening of crops," he says.

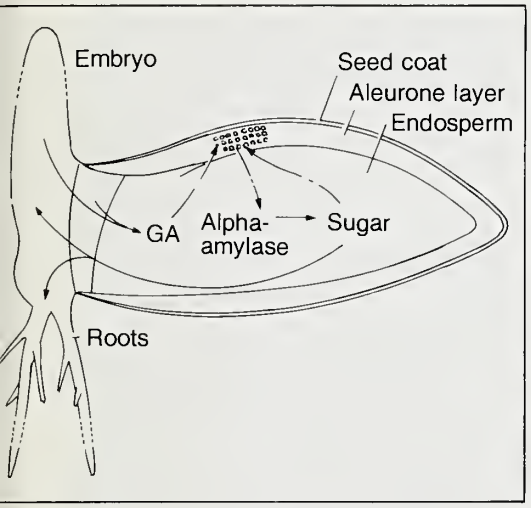
In 1964, Chandra and a colleague found that unless the hormone is supplied to the aleurone cells, no enzyme is produced.

Working with S. Muthukrishnan of Kansas State University, Chandra has now discovered that the gibberellin hormone triggers the formation of specific messenger-RNA (mRNA) molecules, which in turn tell the aleurone cells to produce enzymes, such as alpha-amylase.

Chandra and his colleagues believe that when gibberellic acid enters the aleurone cell after having been released from the embryo, it is somehow "recognized" and combined with a receptor protein outside the aleurone cell's nucleus. Once the hormone and the receptor combine in a protein complex, they are accepted into the nucleus, where the process of producing the alpha-amylase mRNA begins.

Using genetic engineering techniques, Chandra and Muthukrishnan constructed a piece of DNA that is complementary to the enzyme's mRNA. The complementary DNA was inserted into an *E. coli* plasmid and reproduced in the bacteria. Chandra found, however, that the alpha-amylase gene does not produce alpha-amylase enzyme in *E. coli.*

"Our laboratory copy of the gene may be missing a piece of DNA that, in the plant cell, begins the process of mRNA production," says Chandra. "So



further research is needed to understand the expression of foreign genes."

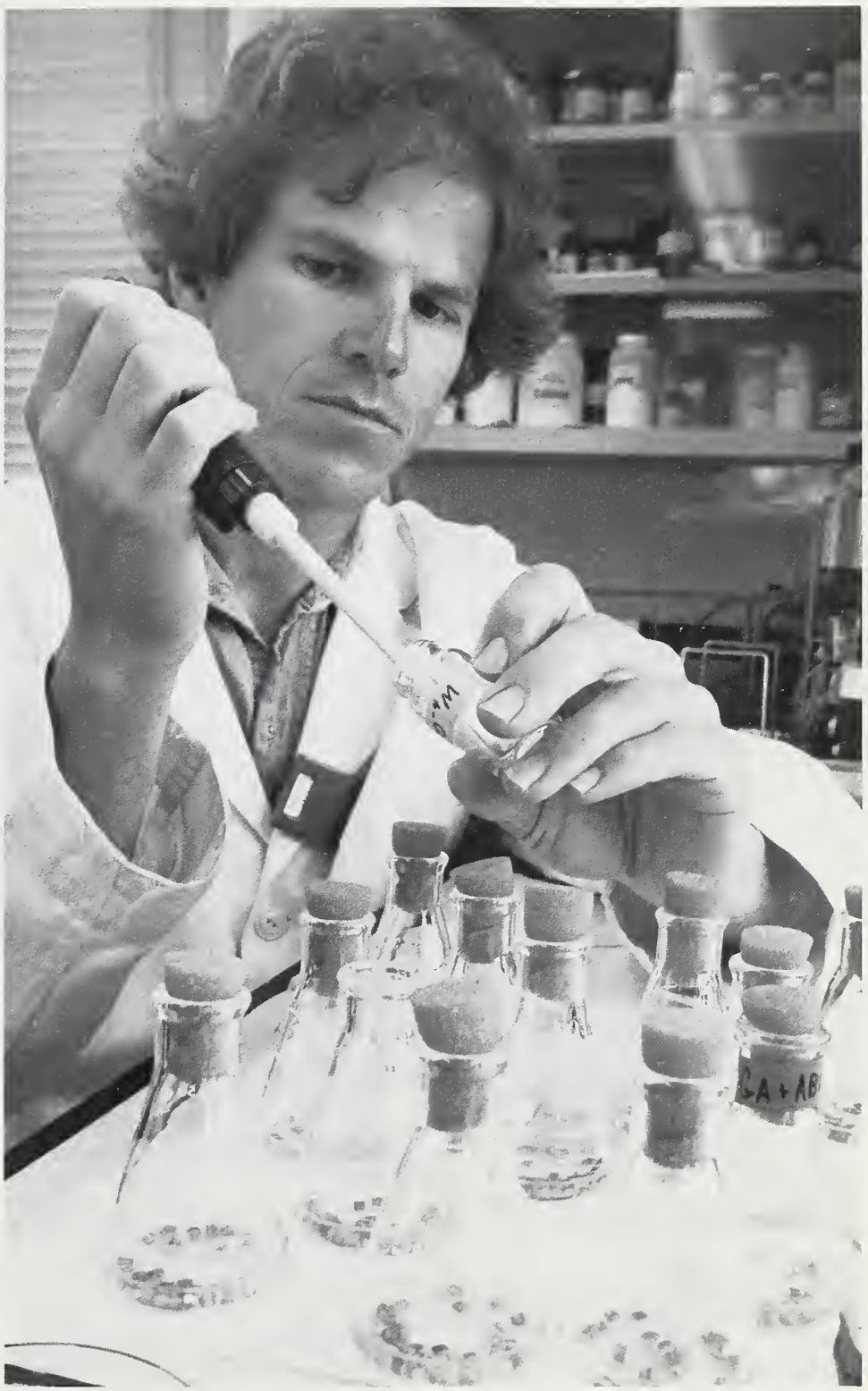
To achieve gene expression, you must know how nature works, Chandra says. Plant cells form the mRNA for alpha-amylase in several stages. First, several plant genes are copied to create mRNA precursors. Then, he speculates, this precursor mRNA may be cut and rejoined to create the mRNA that leads to enzyme production.

"We are using recombinant-DNA techniques to produce an assay tool, or reagent," says Chandra. "The complementary DNA for alpha-amylase, replicated in *E. coli,* can be used to study the process of gene expression in the aleurone cells.

"To create new plants, we will have to learn how specific genes in plants are switched on and off. Discovery of the role of gibberellic acid is such a step.

"A gene inserted into a foreign plant will be dormant and not produce the desired effect unless the proper signals are given for expression of the inserted gene," Chandra explains. "But once a strand of DNA from one plant can be made to express its genetic traits in another plant, genetic engineering will have reached a landmark."

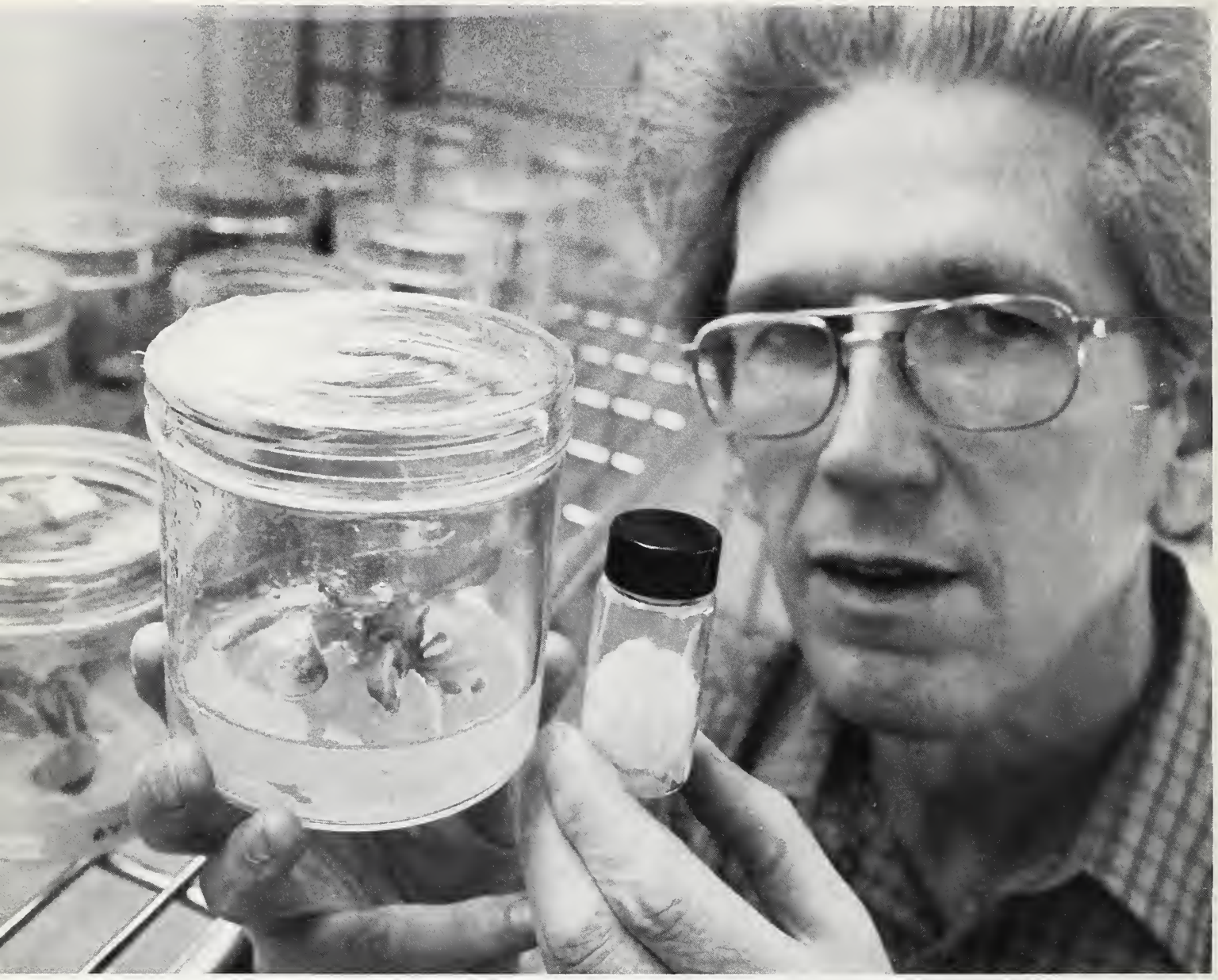
G. Ram Chandra is located at the Seed Research Laboratory, Bldg. 006, Beltsville Agricultural Research Center-West, Beltsville, MD 20705.—(By Ellen Mika and Andy Walker, Beltsville, Md.) ■



Top left: Germinating seed: Gibberellic acid (GA) from the embryo activates aleurone cells to produce enzymes that digest seed starches into sugars for embryo and root growth. (PN6859)

Above: To study the role of gibberellic acid in germination, Albough prepares to apply the hormone to barley seeds. (0782W694-20)

The "Gall" to Cross Mother Nature



Plant physiologist Lowell D. Owens holds a vial (right) of undifferentiated tissue normally produced by tumor cells in culture. The shoots from a mutant tumor cell (left) may advance the regeneration of whole plants from genetically altered cells. (0682X646-9)

Using recombinant DNA techniques, plant scientists have been experimenting with a bacterium as a transfer agent to piggyback genes of one plant species over to another.

Now, ARS scientists have moved the research a step further by regenerating abnormal shoots containing the tiny piece of DNA from the bacterium *Agrobacterium tumefaciens.* Shoot regeneration is essential to any practical application of gene transfer, says Lowell D. Owens, who did the research with Dean Cress and Esra Galun at the Agricultural Research Center in Beltsville, Md.

Agrobacterium tumefaciens can infect about 10,000 species of plants (excluding cereals and other grasses). They cause plants to grow tumors (galls) by transferring a tiny piece of their DNA into the host plant. "This is the only instance known in all of biology where bacteria transfer a piece of DNA into the cells of a higher organism," Owens says. This characteristic holds great potential as a way to take genes for desirable features from one plant and stitch them into another.

The problem, however, has not been in stitching a new gene into the bacterium's DNA or in getting it into the plant cell, but rather in getting the tumor cell to regenerate into a plant. When grown in tissue culture, tumor cells normally divide into masses of

undifferentiated cells which do not form shoots, Owens explains.

Last year, a group of researchers led by John D. Kemp, formerly with ARS, and Timothy C. Hall of the University of Wisconsin, successfully used the bacterium to splice a gene from a French bean into sunflower cells. The new "sunbean" is still a mass of cells growing in tissue culture, however.

In 1981, a group of Belgian scientists, led by Jozef Schell, found a mutant tumor cell that formed normal shoots (like the parent plant). But in the process of mutating, Owens explains, the DNA also lost its ability to form tumors, making it impossible for scientists to recognize the infected cells.

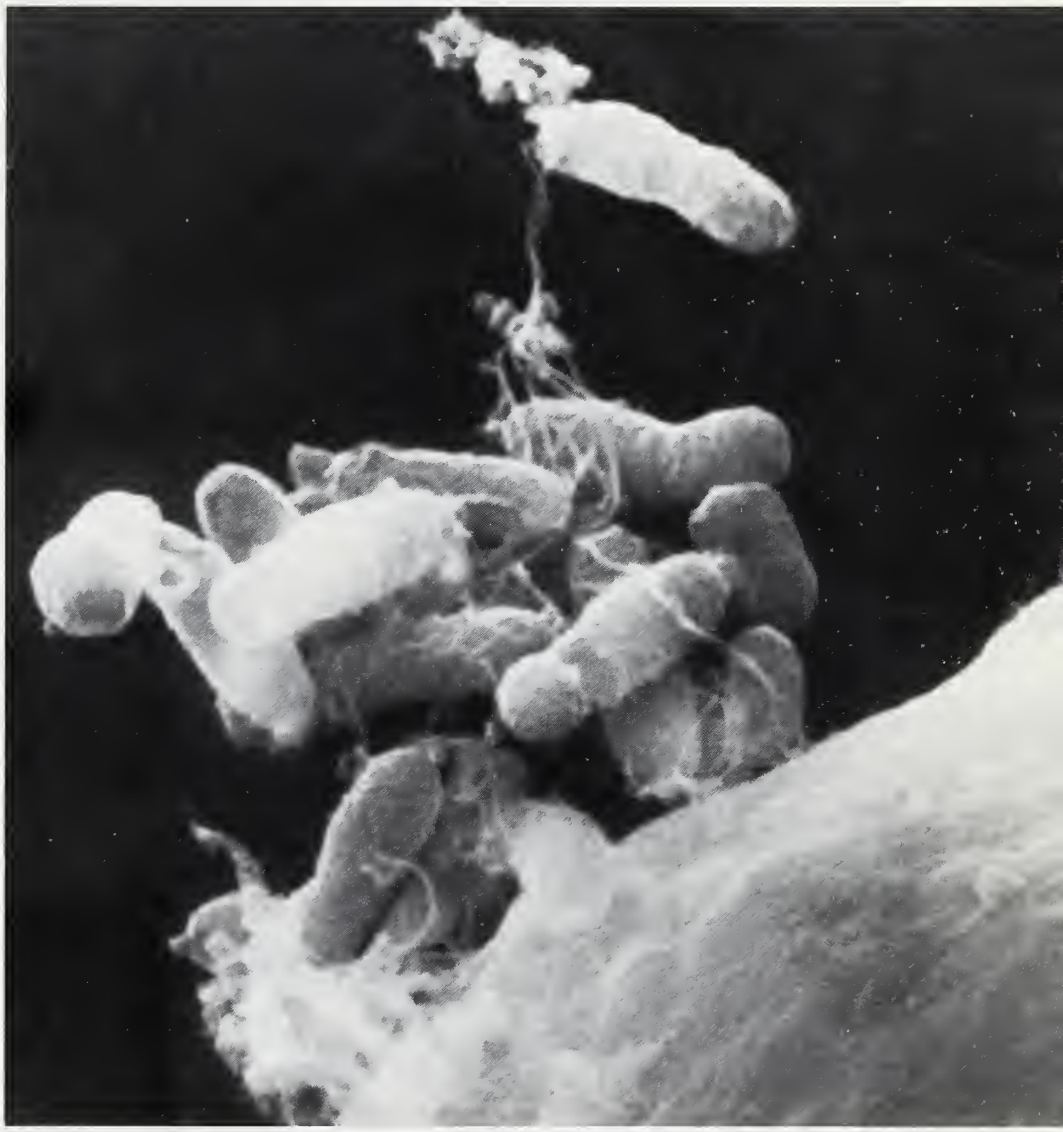
In their experiments, the Belgian scientists discovered that their mutant DNA had lost 1,500 base pairs (the smallest units of DNA). Apparently, Owens says, the genes that cause the plant cells to form tumors lie close to, and possibly interact with, the genes that control regeneration so that the loss of the base pairs affected both characteristics. Or, both characteristics may be controlled by the same genes, he adds.

Owens and his colleagues, during 2 years of growing many thousands of tumor cells in tissue culture, found two that grew shoots. When they cultured cells from one of these shoots, nearly all the cells (62 out of 68) spontaneously grew new shoots. Although the shoots did not resemble the parent plant, the DNA in each cell had been altered in such a way that it normally regenerated.

Owens plans to study the gene sequence in his team's mutant in hopes that "some compromise between the Belgian mutation and whatever we have will produce a tumor cell that will grow into a normal plant."

Owens also sees a possibility of using *Agrobacterium tumefaciens* to transfer genes into species of grasses. Although the bacterium does not currently infect grass plants, it may be able to pass its DNA into grass cells that have had their cell walls removed, he says. This research is still to be undertaken.

Lowell D. Owens and Dean Cress are located at the Cell Culture and Nitrogen Fixation Laboratory, Bldg. 011 A, Beltsville Agricultural Research Center-West, Beltsville, MD 20705. Esra Galun was a visiting scientist from The Weizman Institute of Science, Rehovot, Israel.—(By Judy McBride, Beltsville, Md.) ■



Top left. The presence of crown galls on this tobacco plant is a visible indication that the gene transfer has taken place during infection by *Agrobacterium*. (PN 6855)

Above. Scanning electron microscopy reveals several *Agrobacterium tumefaciens* as they begin to infect a carrot cell. In the process, the bacteria's genetic material will enter the plant cell. (PN 6857) (SEM courtesy of A.G. Matthysse, K.V. Holmes, R.H.G. Gurlitz.)

Test-Tube Rice Yields Better Protein

Plant physiologist Gideon Schaeffer examines high-lysine rice plants grown from tissue-cultured plantlets. Crossbreeding experimental rice with popular varieties may nutritionally improve this major food grain. (0282X100-28)

New rice plants, created in a test tube, will produce grain with 10 percent more protein than other rice varieties. The new plants also provide protein that is more complete for human nutrition. Rice is the third largest food crop in the world.

The new rice has improved nutritional value because it also contains more of the essential amino acid, lysine, than ordinary rice, according to ARS plant physiologist Gideon Schaeffer, Cell Culture and Nitrogen Fixation Laboratory, Beltsville, Md. Amino acids are the building blocks of all the protein in our bodies. Lysine and eight other amino acids are called essential because they must be manufactured from other substances.

"We started with cells grown from rice pollen," says Schaeffer. By chemical selection, we chose cells which had the biochemical potential for improved seed protein. From millions of rice cells, we selected 20 cell clumps for further research.

"Then, in test tubes, these selected cell clumps were grown into plants by using plant tissue culture techniques," he explains. In a carefully controlled environment, the cells were treated with plant growth hormones and were supplied nutrients in specially prepared growing media, Schaeffer says.

When the test-tube plants were large enough, they were transferred into pots of soil, where they produced rice much like normal rice except for its improved protein and lysine content. About 600 plants were then grown in the field.

Rice and other grains are usually low in lysine, a condition that limits the overall nutritional value of rice protein. The lysine deficiency limits the use of the other amino acids by the body, according to Schaeffer.

"By using plant tissue culture techniques we succeeded in finding a high-lysine rice in only a couple of years," says Schaeffer. "Our new selection technique will allow plant breeders to produce new varieties much faster and much more profitably than ever before," he says.

Schaeffer's experimental high-lysine rice plants will be crossbred with popular varieties of rice in an effort to transfer the ability to produce improved protein.

Schaeffer and his associates may be the first scientists to make a significant nutritional improvement in the seed of a major crop through tissue culturing. The technique could open research doors for similarly improving many other food and forage crops, especially when combined with new genetic engineering techniques.

Gideon Schaeffer is located in Rm. 116, Bldg. 011A, Beltsville Agricultural Research Center-West, Beltsville, MD 20705.—(By Ellen Mika and Stephen Berberich, Beltsville, Md.) ■

Anther Culture Speeds Up Wheat Breeding

Tomorrow's amber waves of grain have already taken root in the confines of brightly lit growth chambers. The chambers contain experiments in anther culture, a laboratory system for breeding new crop varieties that could change and speed up the way plant breeders improve wheat.

Anther culture involves generating new plants in test tubes from bits of anthers, the male parts of flowers (also called pollen sacs). The technique bypasses the complex and often unpredictable genetics of sexual reproduction.

Normally, in sexual reproduction in plants, a set of male chromosomes combines with a set of female chromosomes in a flower ovule to form two (diploid) sets of chromosomes in the seed. A plant grown from the seed has a blend of genetic characteristics from both parent chromosome sets. This method of reproduction assures genetic diversity and survival of the species. However, it puts the plant breeder, bent on making a crop improvement, at a disadvantage. Any desirable feature in a sexually produced diploid plant requires long, painstaking inbreeding before the feature is genetically stable and expresses itself reliably in new varieties.

Plantlets which sprout from anther culture carry only a male set of chromosomes. When scientists expose the plantlets to a chemical called colchicine, the chromosomes double. This pairs the plantlet's male chromosomes with duplicates rather than with female chromosomes, as in sexual reproduction. Any desired plant feature is then fixed in the genetic makeup of every cell. Once the plant has grown and matured, it can be self-pollinated to produce identical progeny, or pure breeding lines.

Anther culture saves plant breeders precious time, says research geneticist Stephen Baenziger, who is conducting the experiments with biochemist Gideon Schaeffer at the Agricultural Research Center in Beltsville, Md. "In a 10-year wheat breeding program, for example, inbreeding to obtain pure lines takes the first 5 to 6 years. With anther culture, we get pure breeding lines in 1 or 2 years," Baenziger says.

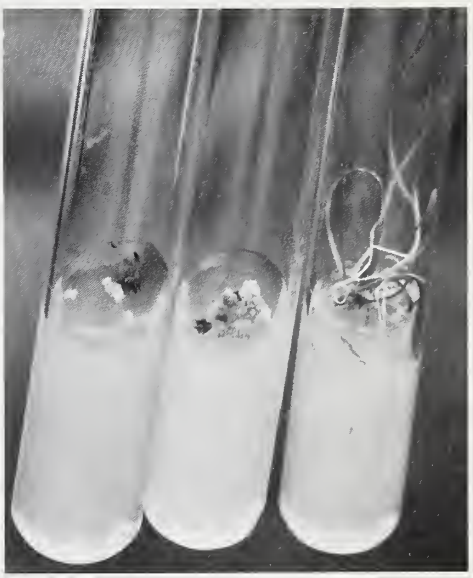
Anther culture also offers another benefit to breeders. Plants propagated from anther culture appear to undergo genetic changes, or mutations, at a far greater rate than occurs in nature or in other means of plant propagation. In greenhouse tests, Baenziger and Schaeffer have already found unexpected variations in anther-generated wheat, such as height differences and changes in seed head structure, that may be desirable traits to breed into future wheat varieties. Anther culture's unique chromosome makeup means that desirable new wheat features, "unmasked by genetic changes from anther culturing, can be captured in pure breeding lines," says Baenziger.

Since 1978, Baenziger and Schaeffer have identified wheat varieties well suited to anther culturing, defined complicated artificial media for test-tube growth, and regenerated numerous types of wheat plants from anthers. They have collected more than a pound of seed from some anther-cultured wheat, and cooperators in Idaho and elsewhere have recently begun field-evaluating plants grown from the seed.

The knowledge and techniques gained from anther culture and other micropropagation systems are essential to the eventual achievement of crop improvement through genetic engineering. Such techniques may make it possible for plant cells containing genetically engineered chromosome changes to be grown into whole, fertile plants.

Baenziger also says that in 4,000 years of wheat cultivation, genetic improvements have evolved slowly. Now, with anther culture, relatively swift breeding explorations are possible of such vast genetic resources as USDA's World Collection of Small Grains in Beltsville, which contains more than 37,000 different wheats and wild relatives.

Stephen Baenziger and Gideon Schaeffer are located at the Beltsville Agricultural Research Center-West, Beltsville, MD 20705.—(By Stephen Berberich, Beltsville, Md.) ■



Top: At a test plot, geneticist Stephen Baenziger (left) and biochemist Gideon Schaeffer check wheat plants grown from anther cultures. The vial contains a young anther-generated plant. (0582W420-23)

Above: White clumps (left) are cells forming from live wheat anthers (dark bodies are dead anthers), (center) masses of undifferentiated tissue grow from the cells; and (right), in a special growing medium, a small plant develops from the cultured tissue. (0682X647-14A)

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Agrisearch Notes

Cotton's Own Bollworm Defenses

Natural resistance to pink bollworm infestation in some varieties of cotton, although not enough to allow cotton growers to stop using pesticides, still offers some benefits.

F. Douglas Wilson, ARS plant geneticist, compared resistant and nonresistant cotton varieties sprayed with an insecticide with the same varieties unsprayed.

The pink bollworm reduced seedcotton, lint, and seed weight 10 to 17 percent more per boll on unsprayed plants than on sprayed plants of a susceptible upland cotton variety and a susceptible pima (long staple) variety.

In an upland cotton variety having some natural pink bollworm resistance, the same yield components were reduced only 1 to 7 percent between the unsprayed and sprayed plants.

Seeds per boll were reduced 7 to 10 percent in the susceptible variety and 4 percent in the resistant one. Bolls per plant were also reduced 8 to 20 percent in the susceptible variety but not at all in the resistant variety.

F. Douglas Wilson is located at the Western Cotton Research Laboratory, 4207 E. Broadway Rd., Phoenix, AZ 85040.—(By Paul Dean, Oakland, Calif.) ■

Date Palm Disease Vector Found

Two ARS entomologists have collected and positively confirmed the presence of *Myndus crudus* Van Duzee, a planthopper suspected of being the insect vector of the lethal yellowing disease of ornamental date palms in the Lower Rio Grande Valley of Texas. It is the same vector that spread the disease through the coconut groves of Jamaica, where it killed trees at a rate of 200,000 per year, and in Florida, where it has killed about one-third of the coconut palms.

The disease itself is a mycoplasma-like organism that has no cell wall, no nucleus, and no internal structures other than DNA and ribosomes. It attacks the phloem tissue of the tree, and death comes when the bud is killed by a soft rot associated with a large number of bacteria that produces a very foul-smelling, putrid odor.

Yellowing of date palms in South Texas was first seen in Brownsville in 1975. Lethal yellowing disease was identified in January of 1980, and entomologist Dale E. Meyerdirk collected the suspected planthopper vector in April of that same year. This was the first report of the insect's occurring in Texas.

To collect the insect, Meyerdirk hung yellow sticky traps in the date palm canopy approximately 15 to 20 feet above the ground. The scientist monitored 74 traps every 2 weeks at 18 different locations throughout the eastern half of the Lower Rio Grande Valley. Meyerdirk also took samples of the insect from small date palms and grasses where the planthopper reproduces. The collected insects were sent to entomologist James P. Kramer, who positively identified them as *Myndus crudus* Van Duzee.

Since the ornamental date palm is found extensively throughout the valley—along highways, roads, and in residential as well as business districts—the esthetic value of this tree is great and its loss would be considerable should the disease not be successfully controlled. Positive identification of the insect vector is a major step leading to the development of control measures.

Dale E. Meyerdirk is located at the Specialty Crops Insects Laboratory, University of California, P.O. Box 112, Riverside, CA 92521. James P. Kramer is with the Systematic Entomology Laboratory, U.S. National Museum of Natural History, NHB 168, Washington, D.C. 20560.—(By Bennett Carriere, New Orleans, La.) ■